A NOTE ON THE CALCULATION OF THE ZONE MOBILITIES OF PEPTIDES FROM THEIR DIFFUSION COEFFICIENTS

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Studies of paper electrophoresis[1] have shown that the zone mobilities u_z of monovalent ammonium ions in a background solution of aqueous acetic acid are given with fair accuracy by the equation

$$u_z = u_o \varrho \eta_o / \eta_a \qquad (1)$$

where u_o is the mobility of the ion in infinitely dilute aqueous solution, η_o and η_a are the viscosities of water and of the aqueous acetic acid solution respectively, and ϱ is an "obstructive factor" characteristic of the paper strip[2,3]. The viscosities at 25° are available in the literature[4], and the obstructive factor may be estimated in various ways[2,3], and tends to a constant value for a given type of paper when the experimental procedure is standardized[1,2]. Hence the possibility of calculating u_z from equation (1) depends on the possibility of calculating u_o. For a relatively small number of ions the equivalent conductances are available from which u_o may be calculated. For the other ions, u_o may in favorable cases be calculated from their volumes, shapes and charges[5]. However, it is sometimes difficult to estimate the shape factor of an irregular ion, or the volume of an excessively hydrated ion such as one derived from a carbohydrate molecule. In such cases it may be possible to calculate u_o from the diffusion coefficient at infinite dilution D_o of the ion, by the equation[6]

$$u_o = zD_oF/RT \qquad (2)$$

where z is the mean ionic charge[7,8], R the gas constant, T the temperature and F the Faraday equivalent.

For zwitterionic compounds such as amino acids or peptides the value of z at different pH values will depend on the extent of protonation of the compound[7,8], and may be calculated from the dissociation constants of the ionizing groups in the molecule. These dissociation constants are available for many simple peptides, and

TABLE I

MOBILITIES IN 30% AQUEOUS ACETIC ACID AT 25°

Compound	z	u_o*	u_z* *(calcd.)*	u_z* *(exptl.)*
Glycylglycine	+0.95	29.3	10.6	10.6
Diglycylglycine	+0.97	25.0	9.1	8.6
Leucylglycylglycine	+0.96**	20.6	7.5	7.4
Insulin	+5.8	37.2	13.5	9.6

* In 10^{-5} cm^2 volt^{-1} sec^{-1}.
** Assuming pK_1 = 3.21.

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may be estimated with reasonable accuracy for others[9]. Values of z calculated in this way for three peptides and for insulin at pH 1.8 are shown in Table I; the exact composition of insulin is now known[10], and the charge shown in the table is calculated for the monomeric form of molecular weight 5750.

The diffusion coefficients in water at 25° of the three peptides shown in Table I, and of many other peptides and amino acids, are known from the meticulous work of LONGSWORTH[11]. These values have been determined at low solute concentrations, and will not differ from D_o by more than 1–2%[12]. The protonation of the zwitterions may be expected to have a very minor effect on their volumes[13], and hence on their D_o values[14]. The values of u_o at pH 1.8, calculated using LONGSWORTH's values of D in equation (2), are given in Table I. The u_o for insulin was calculated from FREDERICQ's[15] diffusion coefficient, obtained by measurements in 20% aqueous dioxane in which the molecule is mainly monomeric.

The zone mobilities of these compounds in 30% (w/w) aqueous acetic acid (pH 1.8, as measured by glass electrode) on Whatman No. 3 paper at 25° were determined following a procedure already described in detail[1], and are shown in Table I. Insulin is known to be monomeric in this solvent system[16]. The absorbance of the paper in this experiment was 1.45 ml/g, so that ϱ was 0.59[3]. In the case of the three peptides, these experimental values of u_z proved to be in reasonable agreement with the values calculated from equations (1) and (2). The experimental u_z for insulin was lower than calculated from these equations, as would be expected for a polyvalent ion[1], but was of the correct order of magnitude.

The success of this correlation would make it appear that the assumptions involved in the theoretical treatment are justified, within the limits of accuracy to be expected

TABLE II

VARIATION IN MOBILITIES IN WATER AT 25° WITH pH

Compound	pH = 1		2		3		4	
	z	u_o*	z	u_o*	z	u_o*	z	u_o*
Glycine	0.96	3.92	0.69	2.82	0.18	0.74	0.02	0.09
Diglycylglycine	0.99	2.58	0.95	2.46	0.65	1.67	0.15	0.40

* Units: 10^{-4} cm^2 volt^{-1} sec^{-1}.

in paper electrophoresis. However, further work is obviously desirable to test the limits of applicability of equations (1) and (2), particularly for ions having $z > 1$. If the equations prove generally applicable to ions having $z \leqslant 1$, they should make it possible to choose optimum conditions for the separation of many amino acids or peptides on a more rational basis than hitherto. Thus amino acids may be made to travel more quickly or more slowly than peptides by controlling the pH of the background solution, as shown by the example in Table II.

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SUMMARY

Zone mobilities of peptides in 30% (w/w) aqueous acetic acid at 25° are in reasonable agreement with values calculated from the diffusion coefficients in water of the neutral compounds.

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